IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Kitto & Burnett	§	**ART UNIT: 1648**
	§	
FILED: February 4, 1999	§	**EXAMINER:**
	§	**Parkin, J.**
SERIAL NO.: 09/244,195	§	
	§	
FOR: Live Vaccine For Human Immunodeficiency Virus	§	**DOCKET: D6073**
	§	

The Assistant Commissioner of Patents
BOX AF
Washington, DC 20231

DECLARATION UNDER 37 C.F.R. § 1.132

Dear Sir:

I, GEORGE BARRIE KITTO does hereby state as follows:

I am a co-inventor of the above-referenced patent application. I have read U.S. patent application serial no. 09/244,195 and I am aware of the content of the Office Action, including all prior art cited against the '195 application.

The following data are presented as evidence showing attenuated *Salmonella* with the HIV epitope-containing plasmids can cause the induction of a cytotoxic CD8 T cell response. Mice were orally immunized twice at 2 week intervals and the cytotoxic response was examined. Spleens were removed from immunized mice and single cell suspensions were prepared. The red blood cells were depleted with lysis buffer (NH_4Cl 8.3 g, $KHCO_3$ 1.0 g, EDTA

0.0372 g/l, pH 7.4). Approximately 5 x 10^6 cells were suspended in Iscoves medium (Gibco) with 10% heat inactivated FCS (Gibco), 50 µl/ml penicillin, 50 µg/ml streptomycin, 2 mM L-glutamine and 5 x 10^{-5} M β–mercaptoethanol in 24 well cell culture plates (Becton Dickinson, UK). To stimulate T cell growth, ConA supernatant (0.5%), methyl α–D-mannopyranoside (50 mM) and HIV-1 reverse transcriptase peptide (0.1 µM) were added to the culture. Spleen cells were stimulated for 5 days. Target cells (RMA/S cells) were incubated with 1 µM HIV-1 reverse transcriptase peptide overnight and then were labeled with 0.2 mCi ^{51}Cr for 45 minutes, washed and resuspended in RPMI, 10% FCS. Target cells were incubated with spleen cells at an effector:target ratio of 10:1. After a 4 hour incubation at 37 °C, cell culture supernatants were collected and radioactivity was evaluated by a γ counter. Specific CTL lysis was expressed as: percentage specific lysis = 100 x (experimental release –spontaneous release)/(maximum release– spontaneous). Maximum release was induced by adding 5% Triton X-100 to wells containing ^{51}Cr-labeled target cells only, while spontaneous release was measured from wells containing target cells in added medium alone. All the CTL assays were performed in triplicate.

The results are shown in Figure 1. Spontaneous release was measured from wells containing target cells in added medium alone (S). Triplicate assays are shown for mice immunized with *Salmonella* expressing either the HIV-pRT (SL3621-RT) or HIVpRTLys (SL3621-RTL) epitopes. The

control group of mice, which were immunized with *Salmonella* containing an unrelated virus epitope plasmid construct, gave negative results. All the mice immunized with *Salmonella* containing the plasmids pRT and pRTLys gave positive results. In all cases the test mice gave a measurably higher degree of lysis of the target cells than did the controls. These results indicate that the live attenuated *Salmonella* vaccines expressing the HIV reverse transcriptase peptide can induce a cytotoxic T-cell response.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or patent issued thereon.

Date: Nov 27 02

Dr. George Barrie Kitto